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REVIEW ARTICLE

Toll-like receptors in the pathogenesis of inflammatory diseases

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Abstract
Toll-like receptors (TLRs) are newly established immune receptors which are critical for host defense through the activation of both innate and adaptive immunity. TLRs can recognize molecules with both microbial and non-microbial origins. Emerging evidence now suggests that TLRs are implicated in the pathogenesis of many chronic diseases, including sepsis, atherosclerosis, ischemia/reperfusion-mediated organ dysfunction, rheumatoid arthritis, diabetes, and cancer. Therefore, an understanding of the role of TLRs in inducing chronic inflammation will provide new insights to help design an effective intervention strategy for inflammatory diseases.

Key words: *Toll-like receptors, inflammation, immunity, myeloid differentiation factor 88, TRIF*

Introduction

Toll-like receptors (TLRs) are germline-encoded receptors that play a pivotal role in detecting invading microbial pathogens and initiating innate and adaptive immune responses (1). While the adaptive immune system requires a fair amount of time to be mature enough to respond to an antigen, innate immunity evokes an instant response to invading pathogens, and thus mounts the first line of defense. TLRs are pattern-recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs) with broad specificities for different microbial components. TLR4, the human homolog of Drosophila Toll, was cloned and shown to activate innate immune cells to express cytokines and co-stimulatory molecules, suggesting that TLRs may mediate the innate immune response to activate the adaptive immune system (2). Antigen-presenting cells (APCs), such as dendritic cells stimulated with TLR agonists, undergo a maturation process to express co-stimulatory molecules such as CD80 and -86, major histocompatibility complex class II molecules, and IL-12 which activate naive T cells to differentiate and proliferate. Some immune cells are activated by TLR agonists to produce cytokines, chemokines, and pro-inflammatory enzymes such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), resulting in the initiation of inflammatory processes. Thus, dysregulation of TLR-mediated signaling pathways can lead to chronic inflammation. In this review, we describe the epidemiological and experimental evidence demonstrating the close relationship between TLRs and certain inflammatory diseases and how the modulation of TLRs influences the pathophysiologic complications.

TLRs recognize molecules derived from both microbial and non-microbial origins

A study (3) involving mice resistant to a Gram-negative bacterial component, lipopolysaccharide (LPS), revealed that hyporesponsive mice have either a null mutation of TLR4 (C57BL/10ScCr) or the replacement of proline with histidine at position 713 (C3H/HeJ), demonstrating that LPS is the agonist of TLR4. Although the limited number of these germline-encoded receptors should deal with a broad spectrum of agonists, different microbial

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components are recognized by different isotypes of TLRs with specificity. TLRs recognize various molecules, including lipids, proteins, and nucleotides. TLR2, together with TLR1 or -6, senses lipopeptides from bacteria. Double-stranded RNA (dsRNA) is recognized by TLR3. TLR5 detects flagellin, a component of bacterial flagella filament. TLR7 and -8 respond to single-stranded RNA and imiquimod compounds. Unmethylated CpG DNA from bacteria and viruses is the agonist for TLR9. Mouse TLR11 responds to urogenital bacterial infection.

Certain TLRs are able to respond to 'danger' signals derived from environmental stress and a damaged host. Fibronectin, hyaluronic acid, heat shock proteins, and fatty acids were shown to be endogenous activators of TLR2 and -4. Necrotic cells are known to activate TLR2. RNA and DNA released from damaged cells can activate TLR7 and -9. A number of studies (4) have shown that lipid molecules can modulate TLR signaling and target gene expression. In mice, a high-fat diet increased nuclear factor (NF)-κB activation and expression of inflammatory genes such as monocyte chemotactic protein-1, colony-stimulating factor, and heme oxygenase-1. However, this induction was greatly diminished in C3H/HeJ mice, which have a nonfunctional TLR4 with a mutation in the cytosolic domain (5). Saturated fatty acids, which are abundant in a high-fat diet, have been shown (6,7) to activate TLR2 and -4. Minimally oxidized low-density lipoprotein (mmLDL) binds to CD14, which is a glycosylphosphatidylinositol-anchored glycoprotein and consequently modifies the cellular responses of macrophages through the activation of TLR4. mmLDL induced the production of cytokines in macrophages partly mediated through the activation of TLR4/MyD88 signaling pathways (8). The expression of inflammatory molecules induced by mmLDL in aortic endothelial cells isolated from C3H/HeJ mice was greatly reduced compared with control mice (9). Oxidized phospholipid components such as oxidized 1-palmitoyl-2-arachidonyl-*sn*-glycero-3-phosphorylcholine (Ox-PAPC) are considered responsible for the stimulating activity of mmLDL (10). Although some other studies (11,12) showed in contrast that oxidized LDL (OxLDL) and Ox-PAPC inhibited LPS activity, these results suggest the significant implication of atherogenic lipid components in modulating TLR4 activity. It has recently been shown (13) that CD36, a scavenger receptor for oxidized LDL and fatty acids, functions as a co-receptor of TLR2/6 dimer for the recognition of microbial diacylglyceride. The association of TLR2/6 with CD36 was induced upon ligand stimulation (14).

Structurally different lipid molecules exert differential regulation of TLR signaling. While Bacterial lipid A which stimulates TLR4 signaling contains saturated fatty acid moiety deacylation or unsaturation of the saturated fatty acid results in the loss of stimulatory activity (15,16). Saturated fatty acids such as lauric acid induce activation of NF-κB and expression of inflammatory genes mediated by the activation of TLR2 and -4. In contrast, unsaturated fatty acids such as docosahexaenoic acid suppress the activation of various TLRs, including TLR2, -3, -4, -5 and -9 (6, 7,17).

The activation of TLRs by endogenous factors is considered an important etiological condition to evoke inflammatory diseases without infection.

Downstream signaling pathways of TLRs

For certain TLRs, dimerization of the receptor is one of the initial steps in the activation of intracellular signaling pathways (18). LPS or lipid A treatment triggers the homodimerization of TLR4 (19). The structural discrimination between different microbial lipopetides of TLR2 is ascribed to the formation of different dimers by combination with TLR1 or -6. Mycoplasmal lipopeptides, which are diacylated, activate TLR2/6 dimer, while bacterial lipopeptides, which activate TLR1/2 dimer, are triacylated (20,21). Therefore, this combinatorial dimerization can enhance the activation of downstream transcription factors and endow the ability to distinguish structural differences in the microbial agonists.

Adaptor molecules are required to transmit receptor activation to the downstream signaling components (Fig. 1). Myeloid differentiation factor 88 (MyD88) is the common and first-described adaptor molecule for most TLRs, with the exception of TLR3 (22). MyD88 contains a Toll/interleukin (IL)-1 receptor (TIR) domain in the C-terminal region and a death domain (DD) at the N terminus. The recruitment of MyD88 to the receptor site is attributed to the homophilic interaction between TIR regions of TLR and MyD88. A DD of MyD88 further associates with a homologous DD of IL-1 receptor-associated kinases (IRAK-4 and -1). This interaction promotes the phosphorylation of IRAK-1 and the consequent interaction with tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6). This leads to the activation of IKK complex and mitogen-activated protein kinases (MAPKs) and ultimately the activation of the transcription factors NF-κB and activator protein (AP)-1 and the subsequent induction of inflammatory and immune-related gene products. Toll-IL-1 receptor domain-containing adapter

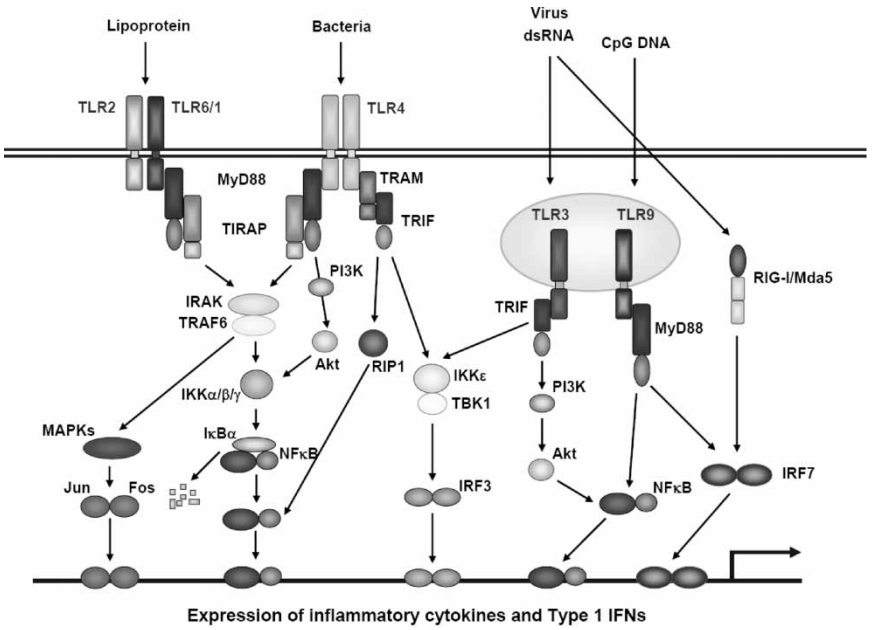

Fig. 1. TLR signaling pathways. Stimulation of TLRs with various ligands leads to the expression of genes that are related to inflammation. TLRs activated by microbial components interact with adaptor molecules such as MyD88 and TRIF which have a TIR domain. The TLR4 signaling pathway comprises both MyD88- and TRIF-dependent pathways, whereas TLR3 is mainly dependent on TRIF. The MyD88-dependent pathway via TLR2 and -4 associates with IRAK and TRAF6, which interact with IKK complex or activate MAPKs. The IKK complex phosphorylates IκBa resulting in nuclear translocation of NF-κB, which can induce inflammatory cytokine expression. In the MyD88-independent pathway, TRIF interacts with IKK-e and TBK1, which are critical molecules for IRF3 activation. The TRIF pathway also leads to the activation of NF-κB in a delayed fashion via RIP1 activity. PI3K and Akt are involved in TLR signaling, enhancing transcriptional activity of NF-κB. TLR9 binds directly to MyD88, inducing IRF7 association. RIG-I and Mda5, activated by dsRNA, trigger IRF7 activation in a TLR-independent manner. IRF7 and -3 are essential for type 1 IFN gene expression.

protein (TIRAP)/myeloid differentiation protein 88 adaptor-like (Mal) is another adaptor which cooperates with MyD88 for full activation of TLR2 and -4 downstream signaling.

The study using MyD88-deficient mice still shows activation of NF-κB and MAPKs in response to LPS, with delayed kinetics suggesting the existence of MyD88-independent adaptors in TLR signaling. Microarray data from LPS-stimulated macrophages isolated from MyD88-deficient and wild-type mice showed that >70% of LPS-induced genes are derived from an MyD88-independent pathway (23). TRIF has been revealed to be responsible for MyD88-independent activation of NF-κB and MAPK. RIP1 is the kinase in the TRIF-dependent pathway responsible for the activation of NF-κB, although the mechanism of how TRIF leads to the activation of NF-κB has not been fully elucidated. The TRIF-dependent kinases TBK1 and IKK-ε phosphorylate and activate IRF3, which is a critical transcription factor for the expression of type I interferons (IFNs) and IFN-inducible genes.

TRIF-related adaptor molecule (TRAM), which is also called TIR domain-containing protein (TIRP) and TIR-containing adaptor molecule-2 (TICAM-2), was discovered to be associated with TLR4, but not other TLRs (24). TRAM interacts with TRIF as an upstream component in TLR4 signaling and is involved in the activation of NF-κB and IRF3. It is notable that different TLRs use different combinations of adaptor molecules. TLR4 has both MyD88- and TRIF-dependent signaling pathways, with the assistance of TIRAP and TRAM. TLR2 recruits MyD88 and TIRAP, but not TRIF, whereas TLR3 mainly activates a TRIF-dependent pathway. TLR5, -7, and -9 have MyD88 as an adaptor.

Phosphatidylinositol 3-kinase (PI3K) and Akt are implicated in various TLR signaling as the downstream kinases involved in NF-κB activation and expression of inflammatory genes such as cytokines and COX-2. NF-κB activation and COX-2 expression, induced by TLR4 agonists such as LPS and saturated fatty acid, were inhibited by LY294002, a PI3K inhibitor and a dominant-negative mutant of PI3K (17). NF-κB activation and cytokine production by CpG DNA, a TLR9 agonist, were attenuated by the PI3K inhibitor wortmannin. The activation of PI3K by LPS or dsRNA (TLR3 agonist) resulted in the phosphorylation of Akt. Akt is a serine/threonine kinase and further phosphorylates its target molecules, including glycogen synthase kinase 3, a pro-apoptotin protein BAD, and caspase-9. Akt is involved in enhancing NF-κB transactivation through p65 phosphorylation. TLR4 does not

interact directly with PI3K. However, LPS induces the association between PI3K and MyD88 in mouse macrophages. A dominant-negative Akt inhibited NF-κB activation induced by constitutively active MyD88, indicating that Akt lies downstream of MyD88. In contrast to TLR4, TLR3 associates with PI3K and this process requires the tyrosine phosphorylation of Tyr759 in the cytoplasmic domain of TLR3 (25). TLR2 stimulation by Staphylococcus aureus induces an association of TLR2 with PI3K and the consequent activation of Akt, leading to NF-κB transactivation (26).

iNOS expression by LPS is dependent on the preceding de novo synthesis of IFN-β, the production of which is mediated through TRIF-dependent signaling pathways (27). IFN-β subsequently acts on IFN-α/β receptor, activating JAK and leading to the phosphorylation of STAT1 (28). Macrophages derived from IFN-α/β receptor knockout or STAT1 knockout mice did not show iNOS expression in response to LPS, indicating that IFN-α/β and STAT1 are required for the expression of iNOS (28). Src-family tyrosine kinases (STKs) also play a role in regulating LPS-induced iNOS expression. The inhibition of STK activity resulted in a reduction in IFN-β, which consequently led to a decrease in iNOS expression, showing that STKs are involved in TRIF-dependent signaling pathways of TLR4 (29). The activation of TLR-related adaptors and downstream signaling pathways culminates in the expression of protein mediators, inducing immune and inflammatory responses (Fig. 2). Enhanced inflammation is, in fact, considered to be a critical etiology for many chronic diseases. Therefore, it is possible that TLR-induced inflammatory mediators can lead to the development of inflammatory diseases. Indeed, accumulating evidence now suggests that TLR-mediated inflammation is implicated in numerous chronic inflammatory diseases.

TLRs and inflammatory diseases

Sepsis

Sepsis is a clinical syndrome associated with bacterial infection. Septic shock represents a severe form of sepsis. Sepsis is accompanied by the activation of systemic inflammatory responses and coagulation pathways. Symptoms become aggravated due to excessive production of nitric oxide and other vasodilators, resulting in low blood pressure and low blood flow, which ultimately leads to multiple organ failure involving the brain, heart, kidney, and liver. Blood can leak from blood vessels into tissues, causing swelling. In particular, leakage and swelling in the lungs can cause respiratory distress. All these septic events eventually result in death. Owing to the increasing incidence rate of sepsis and the associated

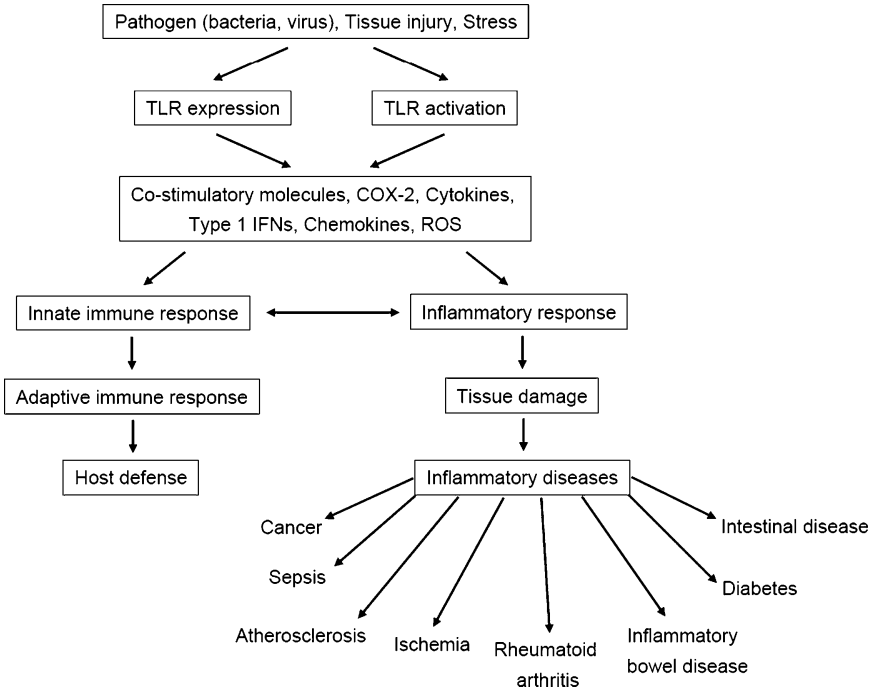

Fig. 2. TLRs are involved in the development of many inflammatory diseases. Various kinds of environmental stimuli affect the level of TLR expression and the activity of TLR. The generation of immune and inflammatory gene products through TLRs leads to the activation of innate and adaptive immune responses as a powerful host defense mechanism. This can also trigger severe inflammatory responses resulting in organ dysfunction and ultimately the development of several diseases. These suggest the critical role of TLRs in modulating innate and adaptive immune responses and the possible implication of TLRs as useful therapeutic targets for inflammatory diseases.

high mortality, it has been considered very important to identify the specific target for sepsis in order to develop effective therapies. Although i.v. injection of high doses of antibiotics has been the primary treatment, this alone is not enough to diminish all the inflammatory processes. Activated protein C and low-dose steroid have been shown to reduce the symptoms. However, the lack of an effective intervention strategy has prompted a continuing search for more specific targets.

LPS derived from the cell wall of Gram-negative bacteria is the major culprit for evoking the symptoms of sepsis by the production of inflammatory mediators such as TNF-α, IL-1β, or IL-8 and the procoagulant tissue factor in macrophages and monocytes. Previously, CD14 had been suspected to be the LPS receptor as it was shown to bind to LPS and LPS-binding protein complex. However, CD14 is not capable of transducing an extracellular signal into the cell because it lacks an intracellular domain. Recently (3), it has been proven that TLR4 is the receptor used by LPS to stimulate widespread inflammation, while CD14 works as a co-receptor. Indeed, LPS-induced impairment of ventricular function was not observed in TLR4-deficient mice (C3H/HeJ), suggesting that myocardial TLR4 contributes to heart failure during septic shock through induction of an inflammatory response (30). This discovery led to a better understanding of the pathogenesis of sepsis and helped to identify new therapeutic strategies for treating sepsis.

Other microbial components, such as lipopeptides, unmethylated DNA, and flagellin, can cause a septic syndrome similar to the effect of LPS. These components activate other TLRs, eliciting an inflammatory process (31). Sepsis can arise in the absence of LPS infection. Many endogenous activators of TLR4, such as heparin sulfate, hyaluronic acid, and heat-shock protein, were suggested to induce sepsis in the absence of infection (32). Soluble heparan sulfate released from extracellular matrix by elastase activates TLR4, inducing an immune response and increasing production of TNF-α, a crucial cytokine in sepsis. Therefore, TLR4 and its signaling molecules will be more useful targets for treating sepsis than LPS itself. Indeed, TLR4 antagonists and inhibitors of TLR4 signaling components are being developed as promising therapeutic agents for the treatment of septic shock (33).

Atherosclerosis

Atherosclerosis is an inflammatory disease characterized by severe immunological events, such as accumulation of atherogenic lipid, inflammatory cell recruitment and production of several chemokines (34). All of these impact atherosclerotic plaque progression. Increased lipoproteins and circulating leukocytes are linked to expression of PRRs, scavenger receptors, and TLRs. Indeed, the expression of TLRs was augmented in macrophages and endothelial cells in human atherosclerotic lesions. In particular, it was demonstrated that expression of TLR1, -2, and -4 in atherosclerotic plaques was associated with NF-κB nuclear translocation (35).

The expression of TLR2 and -4 was also increased in aortic tissues isolated from an animal model of atherosclerosis induced by bacterial infection of invasive Porphyromonas gingivalis (36). In lipid-rich, macrophage-infiltrated atherosclerotic lesions obtained from apolipoprotein E (ApoE)-deficient mice human coronary artery, TLR4 was distinguishably expressed, as determined by immunohistochemical evidence, whereas normal vessels obtained from control mice and healthy human arteries did not show any TLR4 expression (37). This upregulation of TLR4 expression was mediated by OxLDL. OxLDL and mmLDL, which are among the major factors contributing to atherogenesis, are also shown to activate TLR signaling, resulting in the production of inflammatory cytokines.

A knockout animal study showed that TLR signaling was implicated in the pathological development of atherosclerosis. MyD88 null mice fed a high-cholesterol diet have shown a marked reduction in the size of aortic atherosclerotic lesions. Moreover, the mRNA expression of several atherosclerosis-related genes and chemokines, such as monocyte chemotactic protein (MCP) and macrophage inflammatory protein (MIP)-1, is significantly lower in MyD88 knockout mice (38). The results of another study (39) also supported the fact that deficiency of TLR4 or MyD88 led to a significant reduction in atherosclerotic plaque formation during the development of atherosclerosis in ApoE-deficient mice fed a high-cholesterol diet. The degree of macrophage infiltration and the levels of inflammatory cytokines such as IL-12 and MCP-1 in aortic sinus plaques were also reduced.

It has been suggested in numerous studies that the increased risk of cardiovascular diseases such as atherosclerosis is linked to infection with bacteria or viruses which stimulate the innate immune system via TLRs. Intranasal infection of ApoE-deficient mice with Chlamydia pneumoniae resulted in an increase in the area of the atherosclerotic lesion of about twofold compared with control mice (40). The formation of atherosclerotic plaques in heterozygous or homozygous ApoE-deficient mice was accelerated by bacterial infection with invasive P. gingivalis, but not by an invasion-impaired P. gingivalis mutant

(36,41). In addition, repeated exposure to P. gingivalis promoted the development of coronary and aortic atherosclerosis in normocholesterolemic and hypercholesterolemic pigs (42). Although bacterial infection does not seem to be a direct cause of atherosclerosis (43), these studies have shown that pathogen infection can serve as a potentiating factor to exacerbate the progress of atherosclerosis. Further studies demonstrated the activation of TLRs by these bacteria. TNF production by C. pneumoniae in peripheral blood mononuclear cells was greatly attenuated by an anti-TLR2 blocking antibody, while the induction of TNF was not changed in TLR4 mutant macrophages (44). The secretion of chemokines and cytokines such as MIP-1α, MIP-2, TNF, and MCP-1 by C. pneumoniae was diminished in TLR2 knockout mice (45). In TLR2 knockout mice, there was less recruitment of polymorphonuclear neutrophils to the lung following pulmonary infection with C. pneumoniae (45). P. gingivalis enhanced cell adhesion activity of wild-type macrophages to endothelial cells; however, stimulation with P. gingivalis did not increase the binding activity of TLR2-deficient macrophages (46). These results suggest that the aggravation of atherosclerosis induced by C. pneumoniae and P. gingivalis may be closely related to the activation of TLR signaling. These results demonstrate a close relationship between atherosclerosis and innate immunity.

Gene polymorphism studies further support the relevance of TLR activation in atherosclerosis. Asp299Gly TLR4 polymorphism, which shows attenuated receptor signaling with reduced inflammatory responses, is associated with a lower risk of atherosclerosis in humans (47). Another case-control study of patients with acute coronary syndromes (48) confirmed the association between Asp299Gly TLR4 polymorphism and a reduced risk of acute coronary events.

Ischemia/reperfusion-mediated organ dysfunction

Ischemia/reperfusion (I/R) injury is associated with a series of inflammatory processes evoked by reactive oxygen radicals and pro-inflammatory cytokine/chemokine production, which ultimately lead to tissue damage. Accumulating evidence has demonstrated the important role of TLR in the initiation and exacerbation of inflammation and organ dysfunction in several tissues after I/R injury. The contribution of TLR activation in myocardial infarction and heart dysfunction after I/R has been demonstrated in many studies. Myocardial infarct size was greatly reduced in TLR4-impaired mice (C57/BL10 ScCr and C3H/HeJ) after I/R compared with control mice (C57/BL10 ScSn and C3H/OuJ) (49). In TLR4-deficient mice, the levels of inflammatory markers such as infiltrating leukocytes, lipid oxidation, and complement activation in the myocardium were decreased compared with wild-type mice. The activation of NF-κB and AP-1 and subsequent expression of cytokines were significantly diminished in the ischemia-reperfused myocardium of C3H/HeJ mice compared with control mice (C3H/HeN) (50).

Basal TLR4 expression in monocytes obtained from patients with acute myocardial infarction was enhanced compared with that in healthy subjects. This correlated well with the increased plasma cytokine levels in the patients. Responsiveness to LPS was augmented in monocytes derived from patients, as shown by the increased production of cytokines such as IL-6, IL-12, and TNF-α and the co-stimulatory molecule B7-1 (51,52). TLR4 expression in cardiac myocytes was also significantly increased after myocardial infarction induced by ligation of the left coronary artery (53). In addition, TLR2 was shown to be involved in the process of ventricular remodeling after myocardial infarction. Infarct size and the infiltration of polymorphonuclear neutrophils and macrophages into the infarct area did not differ between wild-type and TLR2-deficient mice. However, TLR2-deficient mice showed a higher survival rate, less myocardial fibrosis in the non-infarct area, and reduced expression of transforming growth factor-β_1 and collagen type 1 mRNA than wild-type mice (54).

In contrast, a different role of TLR in cardiac function was reported. The I/R process in tissue is accompanied by oxidative stress, which is one of the critical etiological factors for the pathogenesis of cardiac dysfunction. Hydrogen peroxide-induced activation of NF-κB and AP-1, which has an anti-apoptotic effect in neonatal rat cardiac myocytes, was mediated through the activation of TLR2. Therefore, blockade of TLR2 activity augmented the apoptosis induced by oxidative stress in the heart and resulted in cardiac dysfunction (55). These findings suggest that activation of immune receptors, TLRs, is related to heart failure after acute myocardial infarction and that the regulation of TLR can be a potential therapeutic target for reducing I/R injury. Indeed, i.v. administration of eritoran, a specific TLR4 antagonist, to mice reduced myocardial infarct size after I/R. The effect of eritoran seems to be associated with the downregulation of NF-κB and c-Jun N-terminal kinase (JNK) activation and the decrease in inflammatory cytokine expression (56).

Hepatic I/R injury is accompanied by the increases in the levels of serum alanine aminotransferase (ALT) and cytokines. TLR4-deficient mice exhibited

less hepatic inflammatory response relative to wild-type mice, as shown by a lower serum ALT level and decreased chemokine and cytokine production (57). TLR4 signaling in non-parenchymal cells such as liver macrophages (Kupffer cells) is required for hepatic inflammatory responses and consequent liver failure after I/R injury. Hepatocellular damage after hepatic I/R was greatly attenuated in wild-type mice transplanted with bone marrow from TLR4 mutant mice, while TLR4 mutant mice with wild-type bone marrow cells were not protected from hepatic I/R injury (58).

Renal failure after I/R injury is related to TLR2. TLR2 mRNA expression was markedly enhanced in renal tubular epithelial cells (TECs) after I/R injury. Several chemokines, such as keratinocyte chemoattractant, MIP-2, and MCP-1, were significantly reduced in TLR2-deficient TECs compared with wild-type TECs in vitro and in vivo. The decreases in chemokine and cytokine production coincided with influx of leukocytes. After renal I/R, infiltration of granulocytes and macrophages in the kidney was decreased in TLR2-deficient mice. TLR2 deficiency in the kidney affects renal function, as shown by lower serum creatinine and urea levels. The I/R injury in the kidney was attenuated by TLR2 antisense oligonucleotide (ASON). TLR2 ASON treatment reduced TLR2 protein expression, serum creatinine and urea levels, and the number of apoptotic TECs, resulting in improved renal function. This study demonstrated the importance of TLR2 in the inflammatory response after renal I/R injury and suggested the possibility of a new TLR2 antisense therapy for renal ischemia (59). Together, these results suggest that TLRs play a significant role in I/R-induced tissue damage, as well as in the microorganism-induced inflammatory response.

Rheumatoid arthritis

Rheumatoid arthritis is an autoimmune disease, the pathophysiologic process of which includes chronic inflammation in the joints and requires a complex interaction between different types of cells, such as synovial fibroblasts, macrophages, dendritic cells, T cells, and B cells. Recent studies (60) demonstrated the involvement of the innate immune system in the development and pathological progress of arthritis through the activation of TLRs, resulting in the production of inflammatory cytokines and the recruitment of inflammatory cells. Results from knockout animal studies have shown that a deficiency of TLR4, TLR2, or MyD88 in mice was associated with a decreased severity of arthritic symptoms (61,62). The administration of certain TLR agonists, such as LPS, staphylococcal peptidoglycan, CpG DNA, and dsRNA, to the joints of mice resulted in the development of arthritis, accompanied by an increase in inflammatory cytokines (63–66). The level of certain TLR agonists, such as CpG DNA and bacterial peptidoglycans, was greatly increased in the synovial fluid of patients with rheumatoid arthritis. It is notable that endogenous activators of TLRs, such as heat-shock proteins and necrotic cells, are also implicated in the progress of arthritis, demonstrating the significant role of TLR-mediated inflammation without infection in chronic diseases (67,68). One of the mechanisms by which certain anti-rheumatic compounds exert their effect appears to be related to the regulation of TLR activity. Anti-rheumatic gold compounds, including auranofin and tetrachloroauric (III) acid, suppressed the activation of downstream transcription factors of TLR, IRF3, and NF-κB, and the expression of the pro-inflammatory enzyme, COX-2, which was mediated through the blockade of TLR4 dimerization (69). The suppression of TLR signaling by treatment with inhibitory protein fused with immunoglobulin (sST2-Fc) significantly attenuates collagen-induced arthritis resulting from a reduction in inflammatory cytokine production (70). These results indicate that TLRs play an important role in the development of rheumatoid arthritis, not only by inducing inflammatory signals but also by contributing to the subsequent activation of the adaptive immune system.

Future perspectives: TLRs as anti-inflammatory therapeutic targets

In addition to those described above, a number of pathological complications have been reported to be highly correlated with TLR activation. Results from epidemiological studies (71,72) demonstrated that gene polymorphisms in TLR4 or the TLR6–TLR1–TLR10 gene cluster are linked to the risk of prostate cancer, possibly through the modulation of immune function and inflammation. Saturated fatty acid-induced insulin resistance in muscle is mediated by the activation of TLR2 by fatty acids (73). An antibody or siRNA against TLR2 restored decreased insulin signaling induced by palmitate and suppressed palmitate-mediated IL-6 production. Similarly, the knock-down of TLR4 expression through short hairpin RNA interference(shRNAi) attenuated saturated fatty acid-induced production of inflammatory mediators in adipocytes (74). Furthermore, TLR4-deficient mice showed a tendency to be more protective against saturated fatty acid- or high-fat diet-induced insulin resistance. Modulation of TLR activity is also closely related to the control of various

gastrointestinal inflammatory diseases (75). Therefore, it has now become clear that TLRs, inflammation, and chronic diseases are closely linked to each other. Results from studies with experimental animal disease models and various TLR-deficient mice clearly demonstrated the critical role of TLR activation in initiating and aggravating the symptoms of inflammatory diseases.

Growing evidence suggests that TLRs and their downstream signaling components can serve as good therapeutic targets for treating many chronic inflammatory diseases. One of the downstream kinases, IKK-β, is known to contribute to many diseases through the activation of NF-κB. Another TRIF-dependent kinase, TBK1, is considered to be a critical factor for angiogenesis and tumor growth (76). IFN-β-deficient mice are highly resistant to endotoxic shock induced by LPS challenge, demonstrating the critical role of IFN-β in the cellular response to TLR4 activation (77). Certain TLR antagonists are able to significantly reduce the development or progression of inflammatory diseases. Blockade of TLR dimerization is one of the key mechanisms by which anti-inflammatory agents work (78). Inhibition of TIR interaction with MyD88 resulted in the suppression of cytokine-mediated NF-κB activation (79). Therefore, it will be critical to understnd the cellular mechanisms through which TLR signals are activated in order to provide new opportunities to develop effective therapeutics for chronic inflammatory diseases.

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